DESCRIPTION OF PLATE.

Fig. 1.—Deposit from Tube No. 8. (×250.) After autoclaving for 15 minutes at 105–115° C.
Fig. 2.—Deposit from Tube No. 4. (×250.) Autoclaved previously, 105–115° C.
Fig. 3.—Symmetrically Arranged Deposit from Tube No. 7. (×250.) Not really crystalline. Autoclaved.
Fig. 4.—Coarse Feathery Deposit from Tube No. 17. (×250.) Autoclaved.
Fig. 5.—Fine Deposit from Tube No. 5. (×250.) Autoclaved.
Fig. 6.—Deposit from Tube No. 7. (×250.) Autoclaved.
Fig. 7.—Hyphæ-like Deposit from Tube No. 20. (×240.) Autoclaved.
Fig. 8.—Fine Deposit, Tube 20. (×240.) Autoclaved.
Fig. 9.—Long Looped Fibre from "Colourless" Solution. Metastable silica only. (×440.) Autoclaved.
Fig. 10.—Mixed Deposit in "Yellow" Solution. Metastable silica and ferric hydrate. (×240.) Autoclaved.
Fig. 11.—Hyphæ-like Deposit in Yellow Solution. (×240.) Autoclaved.

The Production of Growths or Deposits in Metastable Inorganic Hydrosols.

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[Plates 2 and 3.]

The results described in the preceding paper conducted me to the study of other methods for obtaining these growths.

The fundamental law established by Pasteur, and now universally confirmed, that organic growth cannot occur in sterilised organic media, leaves a curious hiatus between inorganic evolution and organic evolution.

It is a remarkable historical fact that organic evolution was firmly established a full generation before inorganic evolution, and that, with the exception of certain ingenious hypotheses, the theory and facts of inorganic evolution have only been partially ascertained in late years.

The problem presents two distinct yet closely related lines of attack. One concerns the method by which organic compounds can be built up from inorganic sources, and is more purely a question of energy-transformation; the other is related to the morphology, or minute anatomy, in the region lying between the inorganic and the organic, and deals with the colloidal inorganic forms preceding the organic structures. Energy-transformations, although of

a different type, underlie the morphology also, but the methods of research vary in the two cases.

It is necessary to study how the organic constituents of living organisms with their high content of energy may be formed by inorganic catalysts by utilising other types of energy, such as sunlight; it is also equally important to pay attention to those visible microscopic forms which colloids assume closely resembling the lower living forms, as the region between inorganic and organic is traversed.

It is highly important to be accurate and rigid in such observations, and to realise that they are distinct from the discoveries of Pasteur. It is proven that living organisms are least likely of all to arise in sterilised organic media, but that has nothing to say as to how living organisms first arose, or arise to-day.

The problem is this—What is the link between the organic and the inorganic ?

The forms of growth of crystals have been studied and classified, and it seems reasonable that similar attention should be paid to the forms in which colloids present themselves.

The study of the more complex forms of growth lying between crystals and living organic forms is closely beset with difficulties, on account of the very difficulty of excluding living forms, for one is working here upon the stage which is nearest to acknowledged living types.

The effort in the present research had accordingly been to exclude as far as possible any contamination, and to devise methods of research which would yield only inorganic forms under conditions exclusive of life.

The objective designedly was that of obtaining colloidal growths, and observing how closely the appearances approach those of the lowliest known forms of living organism.

In such a quest, time is an element of the utmost importance. The nature of the colloidal solutions is such that the usual crude procedures of sterilisation are difficult or impossible of application. Antiseptics cannot be employed, because they precipitate the colloid. Heating in an autoclave above the normal boiling point of water, at atmospheric pressure, to 110 to 115° C., often throws the complex inorganic colloid out of solution and activity.

In order to rule out actual life processes events are hastened so as to obtain in a few minutes effects of the same type as those which are usually produced in days or weeks. It is not, therefore, to be expected that the same delicacy and degree of organisation will be obtained as in the slower processes of life, or of metastable colloidal solutions left to themselves for much longer periods.

The difference is similar to the well-known difference in crystallography of the slowly crystallising and the rapidly crystallising solution.

It was pointed out by Graham, as quoted in the previous paper, that time is an essential factor in all colloidal solutions, and that in this respect the colloids approach living organisms.

The first method attempted was that of using stronger solutions of the two reagents of the previous paper, and bringing these together in proportions as close to the point of actual precipitation as possible, so as to hasten operations of growth in order that contamination by organisms might be safely excluded.

For this purpose a 1-per-cent. solution of ferric nitrate and a 1-per-cent. solution of sodium silicate, each in distilled water, were prepared, and from the two a metastable colloidal system was constructed.

In mixing the two solutions the ferric nitrate solution must be taken as the basis and the silicate solution be slowly added to this, for if the reverse procedure be adopted the silicic acid is at once thrown completely out of solution.

If, however, a volume of the ferric nitrate solution be taken, and the sodium silicate solution be added to it drop by drop with constant shaking, it is found that, although every drop produces a precipitate, this, on shaking, redissolves, and no permanent precipitate occurs up to a certain point. Instead, a common solution is formed of colloidal ferric hydrate and colloidal silicic acid.

This point is reached when about 4 c.c. of the silicate solution has been added to 10 c.c. of the ferric nitrate solution. If this point be somewhat exceeded, a brownish-coloured precipitate appears permanently. On examination under the microscope this precipitate is seen to consist of fragments of membrane shrivelled and corrugated and showing thickened tortuous lines like embedded fibres in the substance of the membrane. Short pieces of fibrils project at places, at the borders of the shreds of membrane. The appearance suggests that fibrils are first thrown out of solution, and that subsequently in the meshes of the fibrils more connecting colloid in the gel form is deposited in a thin layer, so forming the membranous shreds of the precipitate.

This view is supported by the appearances shown when the metastable point is not passed. If 4 c.c. of the silicate solution be slowly added with continued agitation to 10 c.c. of the ferric nitrate solution, a slightly opalescent solution is obtained. This shows, on examination microscopically, very few membranes, but a large number of rather coarse fibres, branching and tortuous.

These are not so well developed as those more slowly grown in the

experiments of the preceding paper with the same reagents, but with care fairly well developed networks of fibres may be obtained by this method.

The next method employed was that of mixing two colloidal solutions of opposite cataphoresis (or electrical sign of the colloid), until the point of permanent precipitation was just being approached. The pair of colloids first tested consisted of colloidal ferric hydrate and colloidal silicic acid, each prepared by Graham's method. The two colloidal solutions used were freshly prepared and, when examined microscopically before admixture, were found to be free from fibrils.

In a series of test-tubes varying volumes of the two colloidal solutions were then mixed together, so as always to yield a constant total value of 10 c.c., and the mixture in each case was immediately shaken up and allowed to stand. Thus, in the experiment from which the microphotographs shown in Plate 2, fig. 1, were obtained, a series of tubes were prepared as follows :—Tube No. 1, 9 c.c. colloidal ferric hydrate plus 1 c.c. colloidal silicic acid; tube No. 2, 8 c.c. colloidal ferric hydrate plus 2 c.c. colloidal silicic acid; tube No. 3, 7 c.c. colloidal ferric hydrate plus 3 c.c. colloidal silicic acid, and so on. The colloidal ferric hydrate contained 0·136 per cent. of Fe_2O_3, and the silicic acid approximately 0·1 per cent. of SiO_2, and the above experimental procedure demonstrated that the best admixture for the rapid production of fibrous growths was 8 c.c. of the colloidal ferric hydrate solution to 2 c.c. of the colloidal silicic acid solution.

When mixed in these proportions and then in a few minutes examined under the microscope, magnificent networks are seen which are stained a pale yellow colour in the coarser fibres. Some of the fibres are so delicate and fine that they are only visible with a high magnification and using diminished illumination. Others, such as those shown in the microphotographs of fig. 1, are coarse and easily visible with the low power. Photography of these products is exceedingly difficult, and gives but a poor impression of the networks as seen under the microscope. Some of the medium-sized fibrils are double contoured, they branch, and in many cases show nodulation, cross striations, or divisions. In fact, many of the appearances presented by growing hyphæ are closely simulated.

It has not been possible for me to observe the mode of growth of these fibres. Large numbers are present almost at once when the two colloidal solutions are mixed, for the mixed solution examined straightway with the microscope shows them from the commencement. On careful examination of a freshly mixed preparation microscopically, with the greater part of the illumination cut off, and a magnification of about 400-500 diameters, exceedingly delicate networks and long branching fibrils may often be just

detected, and it would seem probable that the coarser fibres also arise in this way from at first almost ultramicroscopic rows of granules passing out of solution and joining up to form delicate fibrils. These delicate fibrils then thicken up and become more obvious. Looking at the most delicate fibrils actually visible, the eye so slowly takes up the details and one becomes so gradually conscious of the ramifications, that a deceitful impression is easily obtained, that the structures are actually growing under the eye. But on no occasion could I be certain that I had observed actual growth of a network.

A considerable number of observations were taken to determine whether there was any growth at the ends of the coarser fibres, but none could be detected. In many cases where slide and coverslip preparations were preserved for some weeks, there did, however, appear to be an increase in the growths, and appearances were observed of growths which certainly were characteristic and well developed, and had not been seen on earlier examinations of the same preparation. Often, with this pair of colloids, transition stages in the formation of membranes from fibres are to be seen, as is illustrated, for example, on the right-hand side of the lowest photograph in fig. 1. The appearances given by this method of mixing two opposite colloids are exquisitely beautiful and often intricate in design, although they are produced so rapidly.

Also it is important to stress the point that there can be here no contamination effect with adventitious living organisms. Two colloidal solutions are taken, one of a positive, the other of a negative colloid, each solution is examined carefully by the microscope and found to be clear and free from growths, the two are carefully mixed, and there, in a period of a few minutes, are the most delicate and intricately interlaced patterns of long and branching fibrils, as well as growths of coarse fibres.

The next pair of colloidal solutions investigated were ferric hydrate and "colloidal sulphur α" prepared as described by Quincke (*loc. cit.*). The colloidal sulphur α was obtained by taking distilled water previously sterilised by boiling and allowing it to cool. Two portions of the cooled sterile water were taken and saturated respectively with sulphur dioxide and hydrogen sulphide; then to 20 volumes of the sulphuretted hydrogen water was added one volume of the sulphurous acid solution. The result at once is a fine colloidal suspension, or solution, of sulphur particles. This shows microscopically a multitude of fine round granules all in rapid Brownian movement; on standing for about two days the particles show a tendency to grow together into fine coccal-like chains.

The solution may be diluted without precipitating and also may be boiled, it may even be autoclaved at 110° C. for a few minutes with only partial

precipitation, but prolonged autoclaving throws it out of solution. The amount of coagulum when the sulphur passes out of solution demonstrates that there is ultramicroscopic sulphur in colloidal solution in the fresh preparation, in addition to the particles mentioned above. A determination made by evaporating a known volume to dryness and weighing the sulphur gave 0·48 per cent. in the solution as prepared above.

This solution was diluted tenfold before use, as was also the colloidal ferric hydrate solution used for equilibration with it, so that the concentrations of the two solutions as actually mixed were approximately: colloidal sulphur α, 0·048 per cent.; colloidal ferric hydrate, 0·136 per cent.

In order to exclude entirely growths of "organic carbon" organisms, that is "living" organisms, both solutions, while still separate, were put into a steam autoclave at 110° C. for 10 minutes; this caused partial precipitation. The two solutions were then allowed to cool and when cold were mixed as in the following scheme:—

	Test-tube.								
Number	1.	2.	3.	4.	5.	6.	7.	8.	9.
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Ferric hydrate solution.....	9	8	7	6	5	4	3	2	1
Sulphur α solution	1	2	3	4	5	6	7	8	9

There was no complete precipitation in any of the tubes, but a varying amount of increase in the opalescence.

Microscopic examination as rapidly as possible within a few minutes of making the admixtures showed growths in all, but most rapidly and abundantly in test-tubes Nos. 2 and 3. Microphotographs of the growths obtained when 8 c.c. of the colloidal ferric hydrate and 2 c.c. of the sulphur solution were mixed are shown in fig. 2. The growths here again, when sufficiently coarse, give the appearance by a yellowish colour of an iron salt.

The finest threads are only visible in dim illumination. The growths, again, in this case do not yield good microphotographs. All sizes of fibre are seen here from the finest fibrils up to quite coarse fibres, like structures from plants.

Spontaneous Growths in Colloidal Silicic Acid only.—It is well known from the classical experiments of Graham that sufficiently dialysed silicic acid solution is spontaneously metastable, and, after perhaps days, months, or years of keeping, passes out into a solid jelly. It is rather remarkable that

it has not before been examined microscopically at various periods during this metastable existence.

The actual setting into a thick jelly does not appear to yield microscopic structures, probably because this change at its onset is rapid, and so yields only ultramicroscopic structures. But the jelly, at first clear, gradually shows an increasing opacity after setting, and then, if broken up and examined under the microscope, shows flat membranous scales or plates like those described above for the more coarsely precipitated colloidal ferric hydrate.

The most interesting appearances, however, are those found before jelly formation in the perfectly mobile colloidal solution, when the solution is so dilute (1–2 per cent. of SiO_2) that it remains fluid for some weeks.

Since these growths form slowly, as great care as possible was taken, by autoclaving the mother-solutions of sodium silicate and hydrochloric acid and the dialysing apparatus and all glass apparatus and containers, to prevent infection adventitiously from without.

The growths obtained with colloidal silicic acid are illustrated by the microphotographs of figs. 3 and 4. No special care was taken in autoclaving the mother-solutions in the experiment shown in fig. 3, but it is to be remembered that these are strong hydrochloric acid and strong sodium silicate, neither of them suitable media for the growth of organic germs.

After mixing and dialysing, the colloidal silicic acid was run off into a stoppered bottle which had been sterilised by blowing live steam through it. The growths shown were observed after standing for about three weeks at laboratory temperature. The solution contained just over 2 per cent. of SiO_2, had been dialysed for 48 hours, and coagulated in about five weeks' time.

The coarser growths shown in fig. 3, A, B, C, and D, are obtained occasionally when a stronger solution (about 3 per cent.) of colloidal silicic acid is left for two or three days ringed round with gold size. They appear to form when slow massive formation of a gel phase occurs in a fairly strong solution accompanied possibly by slow concentration due to slight evaporation. These forms yield often very beautiful patterns with ramifying and branching fibres.

The coarser fibres seen in the micro-photographs are not cracks in a contracting jelly, but a more solid phase actually growing in a more fluid phase; this is well seen at the growing edge of such a tuft of fibres, as also from fine structural details in the individual fibres.

The appearances seen in fig. 3, E and F, and in all the photographs of

fig. 4, were obtained in a specially devised experiment in which everything for the obtaining of the colloidal silicic acid solution was sterilised beforehand, and then the whole experiment carried out under aseptic conditions.

The dialysis was made in a seamless test-tube of thick parchment paper, measuring approximately 5 cm. in diameter and 20 cm. in length. This dialysing tube at the outset was thoroughly boiled in distilled water, the distilled water used for diluting the solutions and the solutions themselves were also boiled, as also glassware and rubber cork of the apparatus now to be described.

The colloidal silicic acid solution itself when formed cannot be sterilised, as it is by such a process coagulated and thrown out of solution, but the ingredients from which it is made can be autoclaved.

The following disposition of the experiment was accordingly made. A rubber cork, pierced with three holes (through two of which the stems of two glass separating bulbs provided with glass taps passed), was taken and fitted over the mouth of the dialysing tube (5×20 cm.) described above. The dialysing tube was firmly fixed on the rubber cork with thread. Through the third hole in the rubber cork passed a glass tube, bent twice at right angles and narrowed to a fine point at its outer end. The inner end of this tube when in position passed to the bottom of the dialysing tube, and its outer end was either hermetically sealed or sealed with a mercury seal in a small test-tube surrounding its end. The purpose of this third tube was to draw off a sample of the dialysate daily into sterilised test-tubes.

Ten cubic centimetres of distilled water was placed in the dialysing tube. In one separating bulb were placed 20 c.c. of strong, pure hydrochloric acid and 10 c.c. of distilled water; in the other, 13 c.c. of a 38·5-per-cent. solution of sodium silicate and 27 c.c. of distilled water. The upper openings of the two separating bulbs were stoppered with cotton wool.

The whole apparatus, so prepared and filled with the solutions, was placed in a large autoclave and raised by steam to a temperature of 110° C. for a period of 15 minutes. The steam was then shut off and the whole allowed to cool. The apparatus was taken from the autoclave and, by opening the tap on the separator containing it, the hydrochloric acid was allowed to run into the dialysing tube. The sodium silicate solution in the other separator was then allowed to pass in, accompanied by constant shaking.

The dialyser so fitted up was immersed in a large beaker in a running stream of Liverpool tap water. It might be objected that sterilised distilled water ought to have been used here, but a properly sterilised and screened-off supply of cold sterilised distilled water is an exceedingly difficult matter to arrange. So it was determined to rely upon the impermeable properties

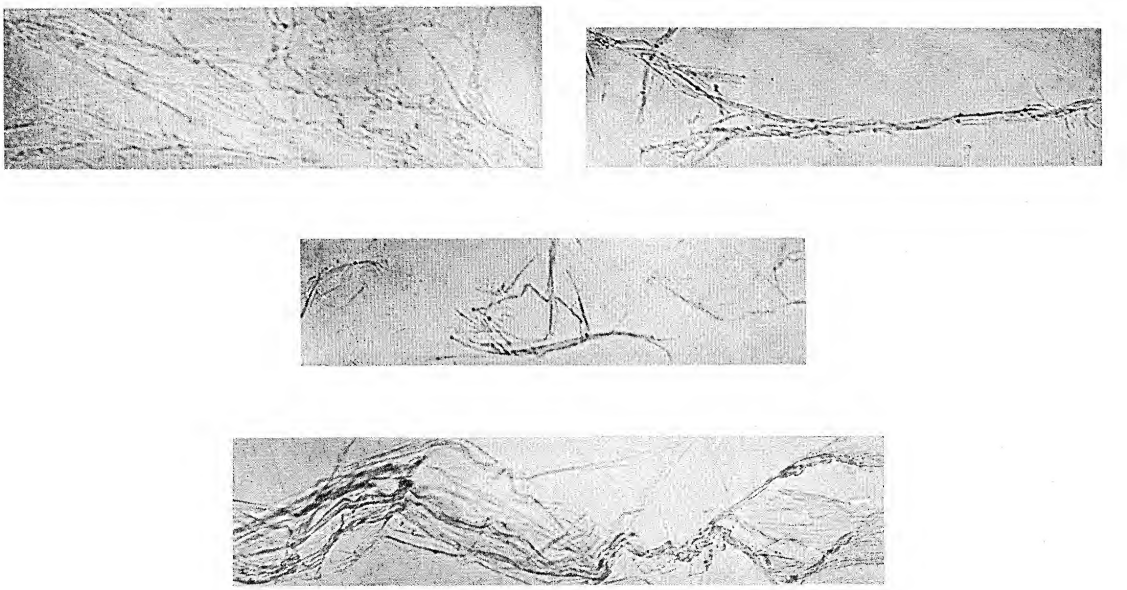

FIG. 1.

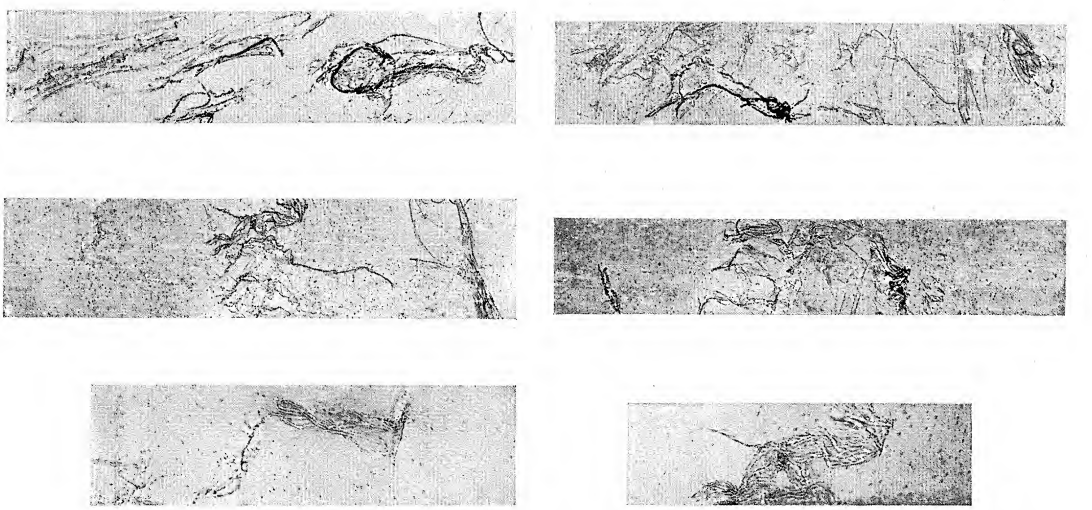

FIG. 2.

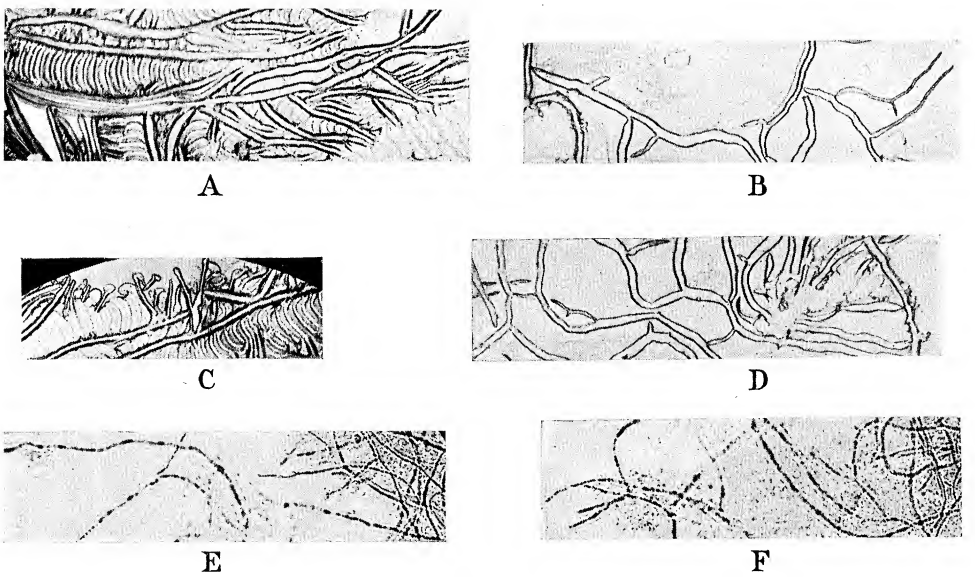

FIG. 3.

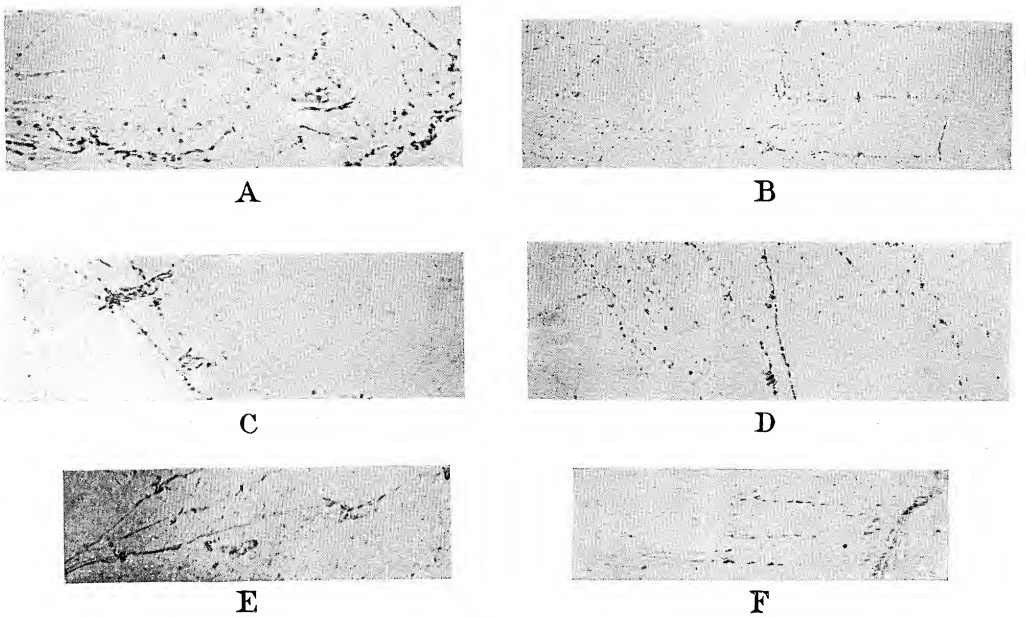

FIG. 4.

for organisms of the autoclaved parchment paper membrane. It is here to be remembered that a stout parchment paper membrane such as was being employed is less permeable than a Chamberland filter. It holds back proteins and inorganic colloids, such, for example, as the silicic acid which it is here being used to separate from the sodium chloride and excess of hydrochloric acid. It is, however, impossible to be quite certain that no chance infection occurred during the experiment, although the tubes did not show any of the usual signs of bacterial invasion.

DESCRIPTION OF PLATES.

Plate 2.

Fig. 1.—Growths formed in Colloidal Solutions in Metastable Proportions of Colloidal Silicic Acid and Colloidal Ferric Hydrate. Magnifications about 240 diameters.

Fig. 2.—Different Views of Growths given by Metastable Admixture of Colloidal Ferric Hydrate and Colloidal Sulphur *a*. The two colloidal solutions, after heating separately to 120° C. for 10 minutes in the autoclave, were mixed in the ratio of 8 of colloidal ferric hydrate solution to 2 of colloidal sulphur *a*, when the growths shown abov appeared in a few minutes. Magnification about 240 diameters in each case.

Plate 3.

Fig. 3.—Coarser Growths and Fibres appearing in Solutions of Colloidal Silicic Acid only. Figs. A, B, C, and D show more rapid growths produced by concentration due to evaporation between slide and cover-slip. Figs. A and C under a magnification of 140 diameters. Figs. B and D under a magnification of 500 diameters. The fibres in all four of these figures are the gel form of the diphasic system. Figs. E and F are forms appearing spontaneously in sterilised and hermetically sealed test-tubes containing colloidal silicic acid prepared aseptically from autoclaved ingredients, as described in the text. The growths were obtained after 30 days' incubation in the sealed tubes at room temperature. Magnifications for figs. E and F, 320 diameters.

Fig. 4.—Finer Forms of Growth appearing in Colloidal Silicic Acid Solution, prepared under aseptic conditions from autoclaved sodium silicate and hydrochloric acid, and kept for 30 days in hermetically sealed glass tubes. Magnification about 240 diameters. These fine nodulated fibrils are very difficult objects to photograph under the microscope, and the micro-photographs only give an idea of their appearance, and fail to reproduce the beauty of the originals.

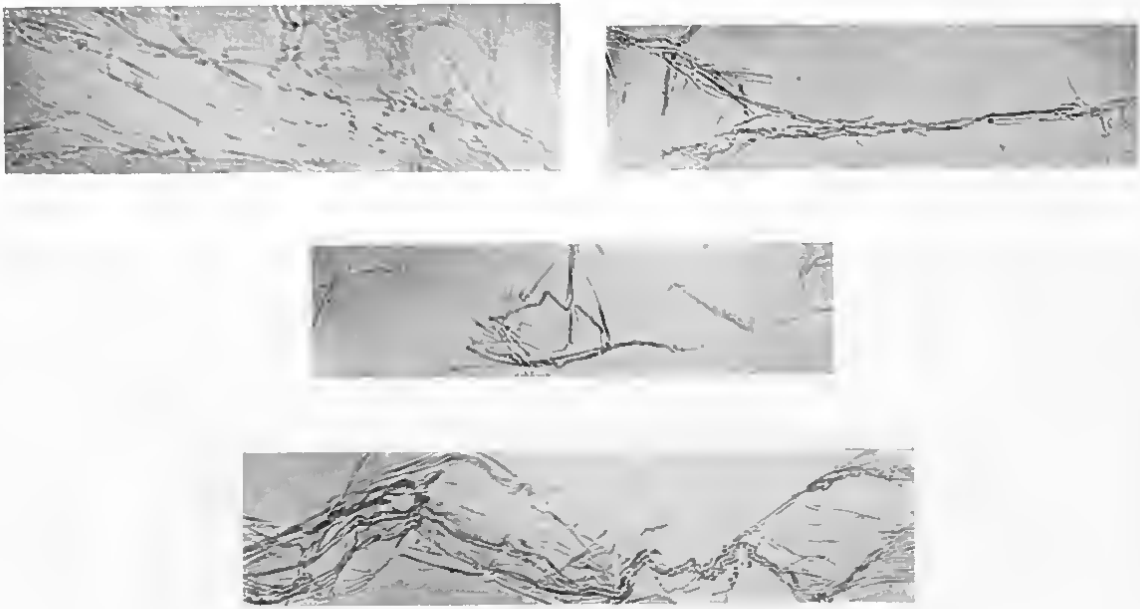

FIG. 1.

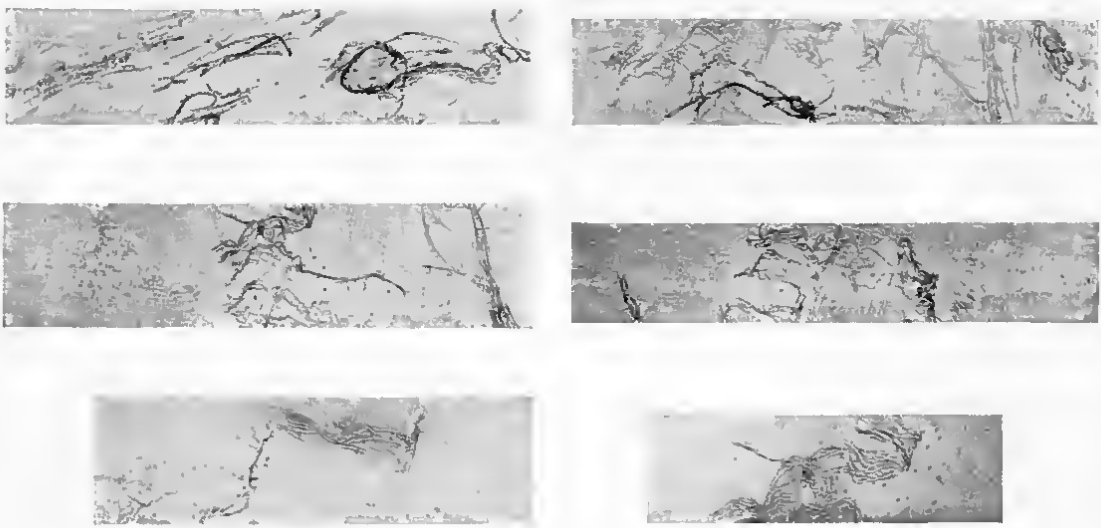

FIG. 2.

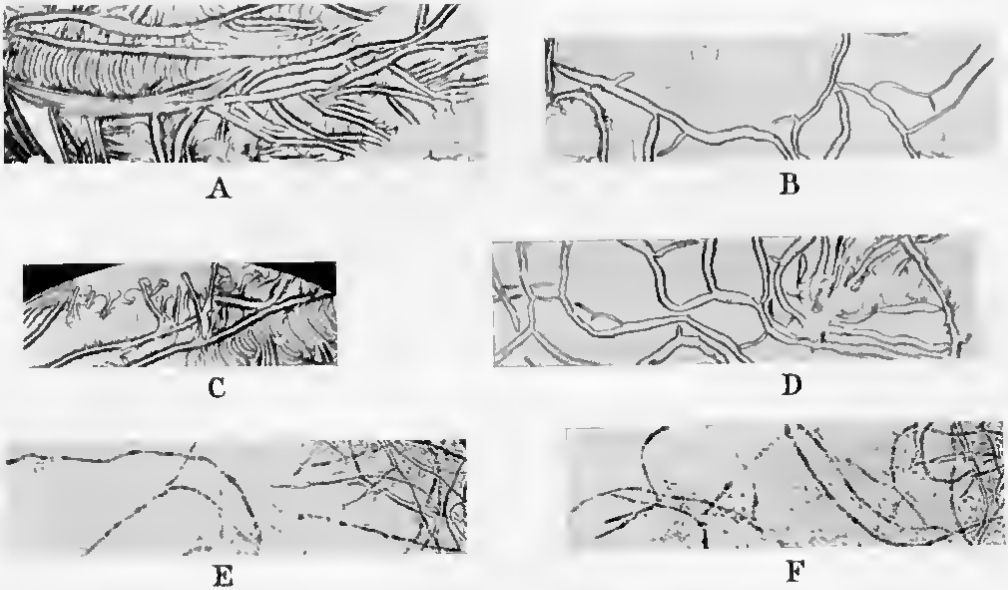

FIG. 3.

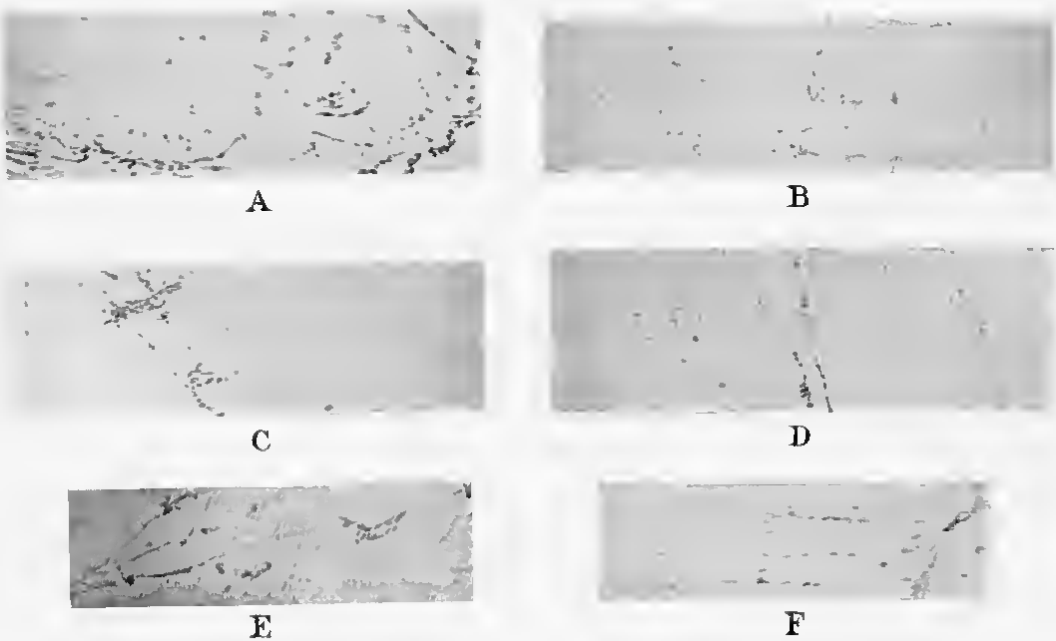

FIG. 4.